Q-219

Assessment of Sewage Pollution in Massachusetts Rivers and Beaches Using a Sewage-Specific Marker
PCR Assay Targeting a Putative Virulence Factor (*esp* Gene) in *Enterococcus faecium*

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INTRODUCTION

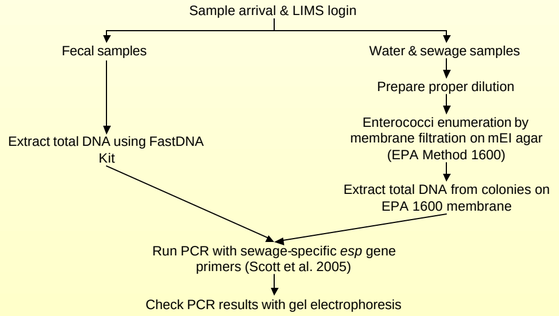
Fecal contamination, as demonstrated by elevated concentrations of fecal indicator bacteria in the water column, is a leading cause of watershed impairment in Massachusetts. Confirmation of the source of fecal pollution in a watershed segment as human (i.e., illicit sewage source) is the logical first step in the development of an appropriate corrective action plan.

Using Massachusetts raw municipal sewage and individual human/animal fecal samples, our laboratory recently demonstrated that the human-specific *Enterococcus faecium* PCR assay targeting the putative virulent enterococcal surface protein (*esp*) gene is highly specific for sewage samples (i.e., generally positive for sewage and negative for individual animal fecal samples). We also did not detect the *esp* gene in 16 individual human fecal samples indicating that not all humans excrete enterococci carrying the *esp* gene.

OBJECTIVES

- Evaluate the sensitivity of the *Enterococcus faecium esp* gene PCR assay using diluted raw sewage samples from 5 Massachusetts municipal wastewater treatment plants.
- Apply the *esp* gene PCR assay to both dry and wet weather samples collected in 2005 from Massachusetts fresh and saline surface waters without permitted sewage discharges.
- Identify relationships between the *esp* sewage marker and other indicators monitored.

METHODOLOGY



- PCR positive and negative controls were run with each sample batch as specified by the U.S Environmental Protection Agency (EPA 815-B-04-001). Our laboratory correctly identified the presence or absence of sewage in 100% of single-blind proficiency test (PT) samples.
- *Escherichia coli* and fecal coliforms were also enumerated in all river water and sewage samples by membrane filtration using EPA Method 1603 and *Standard Methods for the Examination of Water and Wastewater* Method 9222D, respectively.
- Dry weather surface water samples collected from the lower Charles River were also tested for the following sewage-specific chemical indicators: 1) 22 pharmaceuticals, including caffeine, by SPE-HPLC/ESI-MS; and 2) 5 fluorescent whitening agents by SPE-HPLC/FD.

REFERENCE

Scott, T. M., T. M. Jenkins, J. Lukasik, and J. B. Rose. 2005. Potential Use of a Host Associated Molecular Marker in *Enterococcus faecium* as an Index of Human Fecal Pollution. Environ. Sci. Technol. **39:**283-287.

EXPERIMENTAL DATA

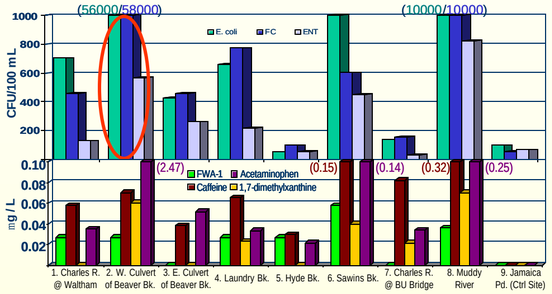


Fig. 1. Enumeration of fecal coliforms, *E. coli*, and enterococci; detection of the *esp* sewage marker (red circle); and quantitation of fluorescent whitening agent #1 (FWA-1), caffeine, 1,7-dimethylxanthine, and acetaminophen in surface water samples from the **lower Charles River** – one dry weather sampling round.

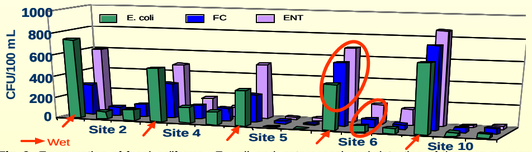


Fig. 3. Enumeration of fecal coliforms, *E. coli*, and enterococci, and detection of the *esp* sewage marker (red circles) in surface water samples from the **Little River (Parker Watershed)** – two dry weather sampling rounds and one wet weather round (red arrows).

Table 1. Sensitivity evaluation of the *esp* sewage marker PCR assay testing varying amounts of total DNA extracted from different dilutions of raw sewage from five Massachusetts wastewater treatment plants (WWTP)

MA WWTP (Mean Flow, mgd[a]) \ Raw Sewage Vol. Tested (mL)	1.0	0.1	0.01	0.001	0.0001	0.00001
	Colony-Forming Units (CFU) per Volume Tested					
	Detection of the *esp* Sewage Marker (P/A)					
Grafton (2.4)	TNTC Present	TNTC Present	TNTC Present	24 Present	1 Absent	0 Absent
Newburyport[b] (3.4)	TNTC Present	TNTC Present	139 Present	5 Absent	4 Absent	0 Absent
Lowell (32)	TNTC Present	TNTC Present	126 Present	17 Present	2 Absent	0 Absent
Upper Blackstone (56)	TNTC Present	TNTC Present	TNTC Present	28 Absent	3 Absent	0 Absent
MWRA Deer Island[b] (1270)	TNTC Present	TNTC Present	56 Absent	11 Absent	2 Absent	0 Absent

[a] mgd: million gallons per day
[b] Raw sewage sample was taken after a week of storm events

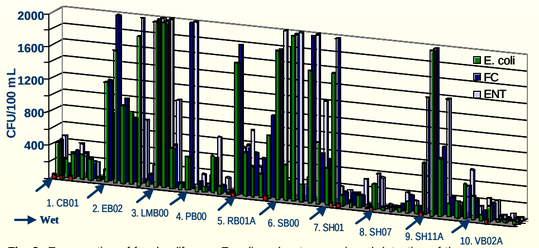


Fig. 2. Enumeration of fecal coliforms, *E. coli*, and enterococci, and detection of the *esp* sewage marker (red bars) in surface water samples from the **Shawsheen River** – five dry weather sampling rounds and one wet weather round (dark blue arrows).

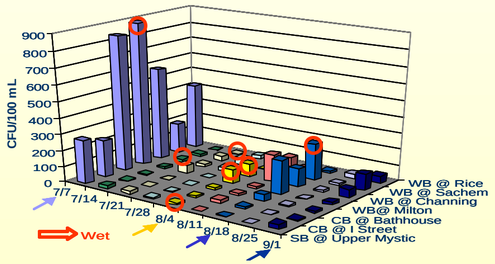
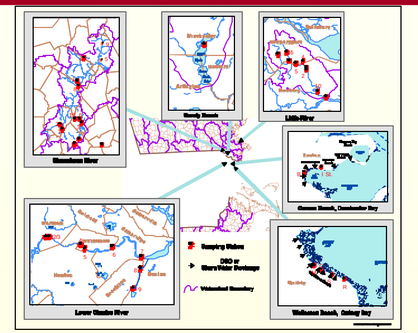


Fig. 4. Enumeration of enterococci and detection of the *esp* sewage marker (red circles) in surface water samples from **Boston-area estuarine and fresh water beaches** – five dry weather and four wet weather (colored arrows) sampling rounds at Sandy Beach/Upper Mystic Lake (SB), Carson Beach/Dorchester Bay (CB), and Wollaston Beach/Quincy Bay (WB).

Table 2. Summary of *esp* sewage marker detections in 2005 from Massachusetts fresh and saline surface waters without permitted sewage discharges

MA Beach / River	Dry Weather Sampling		Wet Weather Sampling	
	Total samples	*esp* Positive	Total samples	*esp* Positive
Beaches				
Carson Beach	10	0	8	0
Salem Beach	13	0	14	0
Sandy Beach	5	0	4	1
Wollaston Beach	20	2	16	4
Rivers				
Little River (Parker)	12	1	5	1
Lower Charles River	14	3	0	--
Shawsheen River	87	7	17	6

SAMPLING STATIONS



RESULTS & DISCUSSION

- Sensitivity evaluation demonstrated that the *esp* sewage marker PCR assay could consistently detect the marker in raw sewage samples from MA municipal wastewater treatment plants at 100-fold dilution (if the sample was collected during wet weather) and up to 1000-fold dilution (Table 1).
- The *esp* sewage marker PCR assay may produce false negatives with water samples containing highly-diluted sewage (>1000-fold dilution of raw municipal sewage) or more concentrated sewage from a single household or a few households with a small number of human individuals that don't carry enterococci with the *esp* gene.
- The *esp* sewage marker was detected in an average of 14% of surface water samples (total data set of 25 out of 180 samples – Table 2) from the following MA rivers and beaches with no permitted sewage discharges, thus indicating the presence of illicit sewage sources: 1) Lower Charles River (1 of 9 samples – Fig. 1), Shawsheen River (10 of 57 samples – Fig. 2), Little River (2 of 15 samples – Fig. 3), Wollaston Beach (6 of 36 samples – Fig. 4), and Sandy Beach (1 of 9 samples – Fig. 4). The marker was not detected in 18 and 27 samples from Carson and Salem Beaches, respectively (see Table 2).
- The *esp* sewage marker was detected in beach water samples with total enterococci concentrations that met as well as in samples that exceeded the corresponding U.S. EPA recreational water quality criterion. In contrast, the *esp* marker was only detected in surface water samples from the lower Charles, Shawsheen, and Little Rivers with enterococci concentrations that exceeded the recreational criterion. However, the *esp* marker was not detected in a significant number of beach and river water samples with enterococci concentrations that exceeded the recreational criterion.
- The *esp* marker was more frequently detected in wet weather than in dry weather samples from the Shawsheen and Little Rivers, Wollaston Beach, and Sandy Beach. For the estuarine Wollaston Beach, there was no correlation between the detection of the *esp* marker in water samples and the tide cycle.
- The *esp* marker was detected along with 4 sewage-specific chemical indicators (i.e., fluorescent whitening agent -1, acetaminophen, caffeine, and 1,7-dimethylxanthine) in a dry weather sample from a lower Charles River tributary thought to be impacted by illicit raw sewage (Fig. 1). Neither the *esp* marker nor any of the sewage-specific chemicals were detected at an upstream control station with no known sewage sources. However, the *esp* marker was not detected in a number of Charles River tributary samples with high levels of indicator bacteria and the presence of two or more sewage-specific chemicals indicating possible *esp* marker false negatives.

CONCLUSIONS

- The *esp* sewage marker PCR assay coupled with the analysis of selected sewage-specific chemical indicators (i.e., fluorescent whitening agents, caffeine, 1,7-dimethylxanthine & acetaminophen) are promising tools for identifying illicit sewage sources in watersheds. However, there is a critical need to increase the sensitivity of the *esp* marker PCR assay in order to decrease its false negative rate.

ACKNOWLEDGEMENTS

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